NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF INDIUM PHOSPHIDE

(CAS NO. 22398-80-7)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

July 2001

NTP TR 499

NIH Publication No. 01-4433

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Information Service (EHIS) http://ehis.niehs.nih.gov (800-315-3010 or 919-541-3841). In addition, printed copies of these reports are available from EHIS as supplies last. A listing of all the NTP Technical Reports printed since 1982 appears on the inside back cover.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF INDIUM PHOSPHIDE

(CAS NO. 22398-80-7)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

July 2001

NTP TR 499

NIH Publication No. 01-4433

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

J.H. Roycroft, Ph.D., Study Scientist
D.W. Bristol, Ph.D.
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
R.R. Maronpot, D.V.M.
D.P. Orzech, M.S.
G.N. Rao, D.V.M., Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S., Integrated Laboratory Systems, Inc.

Battelle Pacific Northwest Laboratories
Conducted studies and evaluated pathology findings

B.J. Chou, D.V.M., Ph.D., Principal Investigator
J.A. Dill, Ph.D.
S.L. Grumbein, D.V.M., Ph.D.
K.M. Lee, Ph.D.
P.W. Mellick, D.V.M., Ph.D.
R.A. Miller, D.V.M., Ph.D.
H.A. Ragan, D.V.M.
Y.F. Su, Ph.D.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
A. Brix, D.V.M., Ph.D.

Dynamac Corporation
Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group
Evaluated slides and prepared pathology report on rats (2 September 1999)

J.C. Seely, D.V.M., Chairperson
PATHCO, Inc.
A. Brix, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
S. Ching, D.V.M., Ph.D.
SVC Associates, Inc.
J. Everitt, D.V.M.
Chemical Industry Institute of Toxicology
S.L. Grumbein, D.V.M., Ph.D.
Battelle Pacific Northwest Laboratories
J.R. Hailey, D.V.M.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
A. Hubbs, D.V.M., Ph.D., Observer
National Institute for Occupational Safety and Health
E.E. McConnell, D.V.M., M.S.
ToxPath, Inc.
A. Nyska, D.V.M.
National Toxicology Program

Evaluated slides and prepared pathology report on mice (19 October 1999)

J.C. Seely, D.V.M., Chairperson
PATHCO, Inc.
A. Brix, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
S. Ching, D.V.M., Ph.D.
Integrated Laboratory Systems, Inc.
J. Everitt, D.V.M.
Chemical Industry Institute of Toxicology
J.R. Hailey, D.V.M.
National Toxicology Program
A. Hubbs, D.V.M., Ph.D.
National Institute for Occupational Safety and Health
E.E. McConnell, D.V.M., M.S.
ToxPath, Inc.
G. Marit, D.V.M., Observer
Battelle Columbus Laboratories
R.A. Miller, D.V.M., Ph.D.
Battelle Pacific Northwest Laboratories
A. Nyska, D.V.M.
National Toxicology Program

Analytical Sciences, Inc.
Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
L.J. Betz, M.S.
K.P. McGowan, M.B.A.
J.T. Scott, M.S.

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
L.M. Harper, B.S.
L.M. Leach, B.A.
D.C. Serbus, Ph.D.
P.A. Yount, B.S.

CONTENTS

ABSTRACT 7

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY 13

TECHNICAL REPORTS REVIEW SUBCOMMITTEE 14

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS 15

INTRODUCTION 17

MATERIALS AND METHODS 25

RESULTS 37

DISCUSSION AND CONCLUSIONS 91

REFERENCES 97

APPENDIX A Summary of Lesions in Male Rats in the 2-Year Inhalation Study of Indium Phosphide 103

APPENDIX B Summary of Lesions in Female Rats in the 2-Year Inhalation Study of Indium Phosphide 149

APPENDIX C Summary of Lesions in Male Mice in the 2-Year Inhalation Study of Indium Phosphide 185

APPENDIX D Summary of Lesions in Female Mice in the 2-Year Inhalation Study of Indium Phosphide 229

APPENDIX E Genetic Toxicology 269

APPENDIX F Clinical Pathology Results 273

APPENDIX G Organ Weights and Organ-Weight-to-Body-Weight Ratios 285

APPENDIX H Tissue Burden Results 291

APPENDIX I Reproductive Tissue Evaluations and Estrous Cycle Characterization 309

APPENDIX J Chemical Characterization and Generation of Chamber Concentrations 313

APPENDIX K Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration 327

APPENDIX L Sentinel Animal Program 331

APPENDIX M Mutations of β-Catenin and H-*ras* in Hepatocellular Adenomas and Carcinomas of B6C3F$_1$ Mice Exposed to Indium Phosphide for 2 Years 335

ABSTRACT

InP

INDIUM PHOSPHIDE

CAS No. 22398-80-7

Chemical Formula: InP Molecular Weight: 145.80

Indium phosphide is used to make semiconductors, injection lasers, solar cells, photodiodes, and light-emitting diodes. Indium phosphide was nominated for study because of its widespread use in the microelectronics industry, the potential for worker exposure, and the absence of chronic toxicity data. Male and female F344/N rats and B6C3F$_1$ mice were exposed to indium phosphide (greater than 99% pure) by inhalation for 14 weeks or 2 years. The frequency of micronuclei was determined in the peripheral blood of mice exposed to indium phosphide for 14 weeks.

14-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were exposed to particulate aerosols of indium phosphide with a mass median aerodynamic diameter of approximately 1.2 μm at concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 by inhalation, 6 hours per day, 5 days per week (weeks 1 through 4 and weeks 10 through 14) or 7 days per week (weeks 5 through 9) to accommodate a concurrent teratology study. One male in the 100 mg/m^3 group died before the end of the study. Body weight gains of all males and females exposed to 100 mg/m^3 were less than those of the chamber controls.

As a result of indium phosphide exposure, the lungs of all exposed rats had a gray to black discoloration and were significantly enlarged, weighing 2.7- to 4.4-fold more than those of the chamber controls. Indium phosphide particles were observed throughout the respiratory tract and in the lung-associated lymph nodes. A spectrum of inflammatory and proliferative lesions generally occurred in the lungs of all exposed groups of rats and consisted of alveolar proteinosis, chronic inflammation, interstitial fibrosis, and alveolar epithelial hyperplasia. Pulmonary inflammation was attended by increased leukocyte and neutrophil counts in the blood. The alveolar proteinosis was the principal apparent reason for the increase in lung weights. Indium phosphide caused inflammation at the base of the epiglottis of the larynx and hyperplasia of the bronchial and mediastinal lymph nodes. Exposure to indium phosphide affected the circulating erythroid mass. It induced a microcytic erythrocytosis consistent with bone marrow hyperplasia and hematopoietic cell proliferation of the spleen. Hepatocellular necrosis was suggested by increased serum activities of alanine aminotransferase and sorbitol dehydrogenase in all exposed groups of males and in 10 mg/m^3 or greater females and was confirmed microscopically in 100 mg/m^3 males and females.

14-WEEK STUDY IN MICE

Groups of 10 male and 10 female mice were exposed to particulate aerosols of indium phosphide with a mass

median aerodynamic diameter of approximately 1.2 µm at concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 by inhalation, 6 hours per day, 5 days per week (weeks 1 through 4 and weeks 10 through 14) or 7 days per week (weeks 5 through 9). Although the effects of indium phosphide exposure were similar in rats and mice, mice were more severely affected in that all males and females in the 100 mg/m^3 groups either died or were removed moribund during the study. One male and three females in the 30 mg/m^3 group were also removed before the end of the study. In general, body weight gains were significantly less in males and females exposed to 3 mg/m^3 or greater compared to those of the chamber controls. Mice exposed to 30 or 100 mg/m^3 were lethargic and experienced rapid, shallow breathing.

As in rats, lungs were discolored and enlarged 2.6- to 4.1-fold greater than those of chamber controls due to the exposure-induced alveolar proteinosis. Indium phosphide particles were observed in the nose, trachea, larynx, and lymph nodes of some exposed males and females. Alveolar proteinosis, chronic active inflammation, interstitial fibrosis, and alveolar epithelial hyperplasia were observed; these effects were more severe than in rats. Hyperplasia in the bronchial lymph nodes and squamous metaplasia, necrosis, and suppurative inflammation of the larynx were observed in some exposed males and females. Exposure to indium phosphide induced a microcytic erythrocytosis which was consistent with the observed hematopoietic cell proliferation of the spleen.

2-YEAR STUDY IN RATS

Groups of 60 male and 60 female rats were exposed to particulate aerosols of indium phosphide at concentrations of 0, 0.03, 0.1, or 0.3 mg/m^3, 6 hours per day, 5 days per week, for 22 weeks (0.1 and 0.3 mg/m^3 groups) or 105 weeks (0 and 0.03 mg/m^3 groups). Animals in the 0.1 and 0.3 mg/m^3 group were maintained on filtered air from exposure termination at week 22 until the end of the studies. Ten males and 10 females per group were evaluated at 3 months.

3-Month Interim Evaluation

Exposure to indium phosphide for 3 months caused a microcytic erythrocytosis and also caused enlarged lungs and lesions in the respiratory tract and lung-associated lymph nodes. Although qualitatively similar to those observed in the 14-week studies, these effects were considerably less severe. However, the lesions in the lungs of rats exposed to 0.1 or 0.3 mg/m^3 were considered sufficiently severe that exposure was discontinued in these groups, and the groups were allowed to continue unexposed for the remainder of the study.

Survival, Body Weights, and Clinical Findings

Exposure to indium phosphide had no effect on survival or body weight gain. During the last 6 months of the study, rats in the 0.03 and 0.3 mg/m^3 groups became lethargic and males breathed abnormally.

Pathology Findings

At 2 years, exposure to indium phosphide caused increased incidences of alveolar/bronchiolar adenomas and carcinomas in rats. Squamous cell carcinoma of the lung occurred in four male rats exposed to 0.3 mg/m^3. As observed in the 14-week study and at the 3-month interim evaluation, a spectrum of inflammatory and proliferative lesions of the lung were observed in all exposed groups of males and females; however, the extent and severity of the lesions were generally greater and included atypical hyperplasia, chronic inflammation, alveolar epithelial hyperplasia and metaplasia, alveolar proteinosis, and interstitial fibrosis.

Exposure to indium phosphide also caused increased incidences of benign and malignant pheochromocytomas of the adrenal gland in males and females. Marginal increases in the incidences of mononuclear cell leukemia in males and females, fibroma of the skin in males, and carcinoma of the mammary gland in females may have been related to exposure to indium phosphide.

2-YEAR STUDY IN MICE

Groups of 60 male and 60 female mice were exposed to particulate aerosols of indium phosphide at concentrations of 0, 0.03, 0.1, or 0.3 mg/m^3, 6 hours per day, 5 days per week, for 21 weeks (0.1 and 0.3 mg/m^3 groups) or 105 weeks (0 and 0.03 mg/m^3 groups). Animals in the 0.1 and 0.3 mg/m^3 groups were maintained on filtered air from exposure termination at week 21 until the end of the studies. Ten males and 10 females per group were evaluated at 3 months.

3-Month Interim Evaluation

Exposure to indium phosphide for 3 months affected the circulating erythroid mass and caused enlarged lungs and lesions in the respiratory tract and lung-associated lymph nodes. These effects, although qualitatively similar to those observed in the 14-week studies, were considerably less severe. However, the lesions in the lungs of mice exposed to 0.1 mg/m^3 and greater were considered sufficiently severe that exposure was discontinued in these groups and the groups were allowed to continue unexposed for the remainder of the study.

Survival and Body Weights

In general, exposure to indium phosphide for 2 years reduced survival and body weight gain in exposed males and females.

Pathology Findings

At 2 years, exposure to indium phosphide caused increased incidences of alveolar/bronchiolar carcinomas in males and alveolar/bronchiolar adenomas and carcinomas in females. In addition to the alveolar proteinosis and chronic active inflammation seen at earlier time points, serosa fibrosis and pleural mesothelial hyperplasia were also present.

The incidences of hepatocellular neoplasms were also significantly increased in exposed males and females. Exposed groups of males and females had increased incidences of eosinophilic foci of the liver at 2 years. Marginal increases in the incidences of neoplasms of the small intestines in male mice may have been related to exposure to indium phosphide. Exposure to indium phosphide also caused inflammation of the arteries of the heart, primarily the coronary arteries and the proximal aorta, and to a lesser extent the lung-associated lymph nodes in males and in females.

TISSUE BURDEN ANALYSES

Deposition and clearance studies of indium following long term exposure of rats and mice to indium phosphide by inhalation were performed. Although there were quantitative differences in lung burden and kinetic parameters for rats and mice, qualitatively they were similar. Deposition of indium in the lungs appeared to follow a zero-order (constant rate) process. Retained lung burdens throughout the studies were proportional to exposure concentration and duration. No differences in elimination rates of indium from the lungs were observed as a function of exposure concentration in either rats or mice. These studies indicated that elimination of indium was quite slow. Mice exhibited clearance half-times of 144 and 163 days for the 0.1 and 0.3 mg/m^3 groups, respectively, as compared to 262 and 291 days for rats exposed to the same concentrations.

The lung deposition and clearance model was used to estimate the total amount of indium deposited in the lungs of rats and mice after exposure to 0.03 mg/m^3 for 2 years or to 0.1 or 0.3 mg/m^3 for 21 or 22 weeks, the lung burdens at the end of the 2-year study, and the area under lung burden curves (AUC). For both species, estimates at the end of 2 years indicated that the lung burdens in the continuously exposed 0.03 mg/m^3 groups were greater than those in the 0.1 or 0.3 mg/m^3 groups. The lung burdens were lowest in the 0.1 mg/m^3 groups. Because of the slow clearance of indium, the lung burdens in the 0.1 and 0.3 mg/m^3 groups were approximately 25% of the maximum levels in rats and 8% in mice approximately 83 weeks after exposure was stopped. The AUCs and the total amount of indium deposited per lung at the time exposure was stopped indicate that the 0.3 mg/m^3 groups were exposed to a greater amount of indium phosphide than were the 0.03 or 0.1 mg/m^3 groups, with the 0.1 mg/m^3 group receiving the lowest exposure. In rats and mice, the second-year AUC for the 0.03 mg/m^3 group was equivalent to that of the 0.3 mg/m^3 group. Regardless of how the total dose of indium to the lung was estimated, total exposure to indium in the 0.1 mg/m^3 groups was less than that in the other two groups implying that in these studies, 0.1 mg/m^3 may be considered the low dose.

GENETIC TOXICOLOGY

No significant increases in the frequencies of micronucleated normochromatic erythrocytes were noted in peripheral blood samples of male or female mice exposed to indium phosphide for 14 weeks. Although there was a significant increase in micronucleated polychromatic erythrocytes in 30 mg/m^3 male mice, there was no increase in female mice, and the percentage of polychromatic erythrocytes was not altered in males or females.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of indium phosphide in male and female F344/N rats based on increased incidences of benign and malignant neoplasms of the lung. Increased incidences of pheochromocytoma of the adrenal medulla in males and females were also considered to be exposure related. Marginal increases in incidences of mononuclear cell leukemia in males and females, fibroma of the skin in males, and carcinoma of the mammary gland in females may have been related to exposure to indium phosphide. There was *clear evidence of carcinogenic activity* of indium phosphide in male B6C3F_1 mice based on increased incidences of malignant neoplasms of the lung and benign and malignant neoplasms of the liver. Marginal increases in incidences of adenoma and carcinoma of the small intestine may have been related to exposure to indium phosphide. There was *clear evidence of carcinogenic activity* of indium phosphide in female B6C3F_1 mice based on increased incidences of benign and malignant neoplasms of the lung. Increased incidences of liver neoplasms in females were also considered to be exposure related.

Exposure to indium phosphide by inhalation resulted in nonneoplastic lesions in the lung of male and female rats and mice, the adrenal medulla of female rats, and the liver and heart of male and female mice.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A Summary of the Technical Reports Review Subcommittee comments and the public discussion on the Technical Report appears on page 15.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Indium Phosphide

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in air	Chamber control, 0.03 mg/m^3 (for 2 years), 0.1 or 0.3 mg/m^3 (exposure stopped at 22 weeks)	Chamber control, 0.03 mg/m^3 (for 2 years), 0.1 or 0.3 mg/m^3 (exposure stopped at 22 weeks)	Chamber control, 0.03 mg/m^3 (for 2 years), 0.1 or 0.3 mg/m^3 (exposure stopped at 21 weeks)	Chamber control, 0.03 mg/m^3 (for 2 years), 0.1 or 0.3 mg/m^3 (exposure stopped at 21 weeks)
Body weights	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group	0.03 and 0.3 mg/m^3 groups lower than chamber control group	Exposed groups lower than chamber control group
Survival rates	27/50, 29/50, 29/50, 26/50	34/50, 31/50, 36/50, 34/50	37/50, 24/50, 29/50, 27/50	42/50, 13/50, 33/50, 21/50
Nonneoplastic effects	Lung: atypical hyperplasia (0/50, 16/50, 23/50, 39/50); chronic active inflammation (5/50, 50/50, 50/50, 50/50); alveolar epithelium, metaplasia (0/50, 45/50, 45/50, 48/50); alveolus, proteinosis (0/50, 50/50, 48/50, 47/50); interstitium, fibrosis (0/50, 49/50, 50/50, 50/50); alveolar epithelium, hyperplasia (11/50, 20/50, 21/50, 31/50)	Lung: atypical hyperplasia (0/50, 8/50, 8/50, 39/50); chronic active inflammation (10/50, 49/50, 50/50, 49/50); alveolar epithelium, metaplasia (0/50, 46/50, 47/50, 48/50); alveolus, proteinosis (0/50, 49/50, 47/50, 50/50); interstitium, fibrosis (0/50, 48/50, 50/50, 49/50); alveolar epithelium, hyperplasia (8/50, 15/50, 22/50, 16/50); squamous cyst (0/50, 1/50, 1/50, 10/50) Adrenal Medulla: hyperplasia (6/50, 13/48, 9/50, 15/49)	Lung: chronic active inflammation (2/50, 50/50, 45/50, 46/50); alveolus, proteinosis (0/50, 14/50, 0/50, 10/50); serosa, fibrosis (0/50, 50/50, 49/50, 50/50) Pleura: mesothelium, hyperplasia (0/50, 19/50, 4/50, 6/50) Liver: eosinophilic focus (10/50, 16/50, 19/50, 18/50) Heart: artery, inflammation (3/50, 18/50, 14/50, 10/50)	Lung: chronic active inflammation (2/50, 49/50, 45/50, 50/50); alveolus, proteinosis (0/50, 31/50, 0/50, 8/50); serosa, fibrosis (0/50, 50/50, 47/50, 49/50) Pleura: mesothelium, hyperplasia (0/50, 16/50, 3/50, 13/50) Liver: eosinophilic focus (6/50, 9/50, 4/50, 12/50) Heart: artery, inflammation (1/50, 16/50, 11/50, 13/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Indium Phosphide

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects	Lung: alveolar/ bronchiolar adenoma (6/50, 13/50, 27/50, 30/50); alveolar/ bronchiolar carcinoma (1/50, 10/50, 8/50, 16/50); alveolar/ bronchiolar adenoma or carcinoma (7/50, 22/50, 30/50, 35/50); squamous cell carcinoma (0/50, 0/50, 0/50, 4/50) Adrenal Medulla: benign pheochromocytoma (10/50, 22/50, 16/49, 23/50); benign or malignant pheochromocytoma (10/50, 26/50, 18/49, 24/50)	Lung: alveolar/ bronchiolar adenoma (0/50, 7/50, 5/50, 19/50); alveolar/ bronchiolar carcinoma (1/50, 3/50, 1/50, 11/50); alveolar/ bronchiolar adenoma or carcinoma (1/50, 10/50, 6/50, 26/50) Adrenal Medulla: benign pheochromocytoma (2/50, 6/48, 2/50, 9/49)	Lung: alveolar/ bronchiolar carcinoma (6/50, 15/50, 22/50, 13/50) Liver: hepatocellular adenoma (17/50, 24/50, 23/50, 32/50); hepatocellular carcinoma (11/50, 22/50, 23/50, 16/50); hepatocellular adenoma or carcinoma (26/50, 40/50, 37/50, 39/50)	Lung: alveolar/ bronchiolar adenoma (3/50, 6/50, 10/50, 7/50); alveolar/ bronchiolar carcinoma (1/50, 6/50, 5/50, 7/50); alveolar/bronchiolar adenoma or carcinoma (4/50, 11/50, 15/50, 14/50) Liver: hepatocellular adenoma (12/50, 14/50, 18/50, 14/50); hepatocellular carcinoma (6/50, 17/50, 8/50, 10/50); hepatocellular adenoma or carcinoma (18/50, 28/50, 24/50, 23/50)
Uncertain findings	Skin: fibroma (1/50, 4/50, 7/50, 3/50) Mononuclear Cell Leukemia: (16/50, 23/50, 29/50, 25/50)	Mammary Gland: carcinoma (0/50, 8/50, 3/50, 2/50) Mononuclear Cell Leukemia: (14/50, 21/50, 14/50, 24/50)	Small Intestine: carcinoma (0/50, 1/50, 5/50, 3/50); adenoma or carcinoma (1/50, 2/50, 6/50, 3/50)	
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence
Genetic toxicology				
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:			Negative	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on indium phosphide on 18 May 2000 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

A. John Bailer, Ph.D., Chairperson
Department of Mathematics and Statistics
Miami University
Oxford, OH

James S. Bus, Ph.D., Principal Reviewer
Health and Environmental Sciences
Dow Chemical Company
Midland, MI

Linda A. Chatman, D.V.M.
Pfizer, Inc.
Groton, CT

John M. Cullen, Ph.D., V.M.D., Principal Reviewer
Department of Microbiology, Parasitology, and Pathology
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

Harold Davis, D.V.M., Ph.D.*
Director of Toxicology
Amgen, Inc.
Thousand Oaks, CA

Norman R. Drinkwater, Ph.D.
McArdle Laboratory for Cancer Research
University of Wisconsin-Madison
Madison, WI

Susan M. Fischer, Ph.D.*
M.D. Anderson Cancer Center
The University of Texas
Smithville, TX

Stephen S. Hecht, Ph.D.
University of Minnesota Cancer Centers
Minneapolis, MN

Michele Medinsky, Ph.D., Principal Reviewer
Durham, NC

Jose Russo, M.D.*
Fox Chase Cancer Center
Philadelphia, PA

---

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 18 May 2000, the draft Technical Report on the toxicology and carcinogenesis studies of indium phosphide received public review by the National Toxicology Program's Board of Scientific Counselor's Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.H. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of indium phosphide by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. Additionally, tissue burden (lung deposition and clearance) studies were conducted in rats and mice from the 14-week and 2-year studies. The proposed conclusions for the 2-year studies were *clear evidence of carcinogenic activity* in male and female F344/N rats and B6C3F$_1$ mice.

Dr. Medinsky, a principal reviewer, agreed with the proposed conclusions. She stated that the report was well written and was based on a well-designed study. Dr. Medinsky added that the discussion section of the report excelled in relating the results of these studies to what is known regarding the mechanisms of action of other lung carcinogens. She further stated that the deposition and clearance studies of indium phosphide in the lung and the toxicokinetic model developed from those studies proved to be extremely valuable for relating neoplasm incidences to the actual exposure of indium phosphide in the lungs.

Dr. Cullen, the second principal reviewer, agreed with the proposed conclusions. However, he thought that *some evidence of carcinogenic activity* might be more appropriate for the findings on liver neoplasms in male and female mice in view of limited exposure-related responses and the fact that neoplasm incidences were similar to historical control rates (for mice fed other diets). Dr. J.K. Haseman, NIEHS, said the liver neoplasms in mice could be dealt with in a manner analogous to pheochromocytomas in rats, i.e., "The increased incidences of liver neoplasms in males and females were also considered to be exposure related." Dr. Cullen suggested that since the mechanism of injury for indium phosphide is not known, greater discussion of the significance of grouping the animals on the basis of the exposure concentration or the total lung burden and the effects of the duration of exposure might be useful. Dr. Roycroft responded that he would try to clarify references to continuous versus stop exposures in the Results and Discussion and Conclusions section. Dr. Haseman explained that in terms of the statistical analyses, no attempt was made to rank the continuous exposures versus the stop exposures, and that the exposure-response trends reported were based strictly on the chamber control and two stop-exposure groups.

Dr. Bus, the third principal reviewer, agreed with the proposed conclusions. He commented that the analyses of tissue concentrations of indium phosphide were a valuable component of the study, with the information providing a more accurate assessment of internal dosimetry as well as confirming that the exposures, despite causing pulmonary neoplasms, probably did not result in pulmonary particle overload.

Dr. Cullen commented that he had trouble trying to compare the discontinuous and continuous exposed animals as to whether there was a clear exposure-related effect. Dr. Roycroft noted that although the external exposure of the two higher exposed groups was only 21 or 22 weeks, the tissue clearance of indium phosphide was extremely slow such that at the end of two years about 25% of the deposited material remained in the lung. Dr. Bailer said that he would like to have an idea of the precision associated with area under the curve (AUC) estimates, such as standard errors. Dr. Medinsky speculated that during the 2-year exposure period, the earlier exposures might be more important and thought the important dosimetric might be some weighted AUC giving more weight to the earlier exposures. Dr. Cullen stated that he still had trouble including liver neoplasms in mice under *clear evidence* in that they were treatment-related but not exposure-related effects. Dr. Bus suggested using the wording mentioned by Dr. Haseman.

Dr. Medinsky moved that under the conditions of this study the Technical Report on indium phosphide be

accepted with revisions discussed and the conclusions as written for male and female rats and mice, *clear evidence of carcinogenic activity*, except that in mice, the citation for liver neoplasms would be included in a separate sentence to read: "The increased incidences of benign and malignant neoplasms of the liver in males and females were also considered to be exposure related." Dr. Cullen seconded the motion. Dr. Haseman pointed out that the trend test for hepatocellular adenomas in males was quite significant and the incidences of hepatocellular carcinomas in males were increased. Dr. J.R. Hailey, NIEHS, affirmed that there was a much stronger response in males. Dr. Medinsky asked that her motion be amended to retain the citation for liver neoplasms in male mice under *clear evidence*, while leaving the citation for liver neoplasms in female mice in the separate sentence. Dr. Cullen agreed to this change. The revised motion was accepted unanimously with six yes votes.